—Note—

A case of equine piroplasmosis in the Tokyo 2020 Olympic Games

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Equine piroplasmosis is an infectious disease caused by Babesia caballi and Theileria equi. A competition horse that had been imported to the Equestrian Park for the Tokyo 2020 Olympic Games and had a fever over 40°C and severe anemia was diagnosed with equine piroplasmosis by blood smear and direct polymerase chain reaction (PCR) tests for Theileria equi. Treatment with protozoan anthelmintics and whole blood transfusion diminished the fever, improved the anemia, and allowed the horse to return home safely. Preparation for routine cases of this infection should include the development of a system that allows accurate and prompt international dissemination of information and implementation of quarantine measures.

Key words: *equine, imidocarb, packed cell volume, piroplasmosis*

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Equine piroplasmosis (EP) is a tick-borne hemoprotozoan disease of equids caused by *Babesia caballi* and *Theileria equi* [4, 5, 9, 13]. *Theileria equi* is generally considered to be more virulent than *Babesia caballi*, with a fatality rate of 10% [14], and affected horses show fever and anemia. The incubation period is 10–30 days for *Babesia caballi* and 12–19 days for *Theileria equi* [4, 7, 13].

EP is classified as a livestock infectious disease in Japan's Act on the Prevention of Infectious Disease in Livestock. Generally, an EP inspection is carried out at the time of import quarantine when a horse is imported into Japan. If a horse is found to be positive for EP, entry into Japan is prohibited, and strict measures, such as returning the horse to the country of origin or slaughtering it, are deployed. Because it was known that many EP-antibody-positive horses would participate in the Tokyo 2020 equestrian event, the event venue was designated as a quarantine facility, allowing horses to compete but not leave the facility. EP-antibody-positive horses were therefore able to participate in the Olympic and Paralympic Games. As screening tools within 30 days of import into Japan for the Olympics, indirect fluorescent antibody tests (IFATs) for *Theileria equi* and *Babesia caballi* were recommended [3].

Treatment of competition horses is guided by the Fédération Equestre Internationale (FEI) [12]. Therefore, treatment on the day of the competition is limited because some drugs are prohibited during and before competition. In addition, some drugs must be reported to the Veterinary Delegate if used within the competition venue. In the present case, fever was confirmed before the competition [12].

EP is recognized as an exotic condition in only a few countries/territories, including Japan, USA, Canada, and New Zealand. In Japan, EP was detected in horses imported from France during an import quarantine in 2016. Except on the premises of the Animal Quarantine Service, Japan has never had antibody-positive horses that could serve as asymptomatic carriers of this disease.

Herein, we report the successful management of a horse with piroplasmosis that was imported as antibody negative and detail the management procedure.

The patient in this case was a 15-year-old German Warmblood gelding. The horse arrived in Tokyo on July 21, 2021, and it was normal on physical examination upon arrival. Due to the Olympic and Paralympic competition, the

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Japan Racing Association Equestrian Park at Bajikoen was designated as an equine disease-free zone (EDFZ), and all the participating competition horses were quarantined for seven days in Germany or Australia before being imported. Upon further investigation after this infected horse became ill, it became apparent that the horse had a fever for one day while in quarantine in Aachen, Germany, before transport to Tokyo on a flight originating in Liege, Belgium. Its complete blood count (CBC) at the time of fever in Germany showed normal results, and its temperature returned to normal without any therapeutic intervention the following day in Germany before export quarantine. Pre-export testing for EP using an IFAT showed negative results.

On the morning of July 24, the horse was observed to be lying down, and colic (abdominal pain) was suspected by the rider and team veterinarian. On physical examination, the horse had a rectal temperature of 40.4°C (Table 1 and Fig. 1, blue line). The heart rate, 64 beats/min, was elevated. The horse had a grade 4/6 decrescendo diastolic murmur, which was suspected to be an aortic insufficiency common in older horses and of minimal athletic importance. The respiratory rate was 20 breaths/min with normal sounds on auscultation. Gastrointestinal sounds were minimal and overnight stool was normal. No digital pulses were noted. Wind puffs (tendon sheath fluid accumulations) were found in the hind fetlocks. After the initial physical examinations, the horse received ketoprofen at 2.2 mg/kg intravenously (IV; Table 1) for the 40.4°C fever (Fig. 1, blue line), because the first day of competition (Horse Inspection) was scheduled for July 28 and the ketoprofen withdrawal threshold for the FEI is 3 days. The FEI withdrawal thresholds for flunixin meglumine (FM) and phenylbutazone were both 6 days, and administration of these drugs could have triggered a positive drug test if the horse recovered quickly from the fever and remained able to compete.

In the initial CBC, the packed cell volume (PCV) was 16.6% (Table 1 and Fig. 1, red line), hemoglobin concentration was 6.5 mg/d*l*, red blood cell count was 3.88 million/μl, and white blood cell count was 7,450/μl. Plasma chemistry showed normal findings, except that the total bilirubin level was 7.7 mg/d*l*. Changes in the blood test results are shown in Table 2, and changes in the rectal temperature and PCV are shown in Fig. 1. Ultrasonographic examination of the thorax revealed normal findings. Abdominal ultrasound also showed normal findings, except for the presence of significant splenomegaly. Owing to the low PCV, the CBC was repeated, and it continued to show a low PCV of 16.4% (Table 1). Because of the documented low PCV and fever, we recommended that the horse be withdrawn from the competition, assessed further, and treated.

At 0930 hr on July 24, empirical therapy was initiated with FM at 2.2 mg/kg IV BID and dexamethasone at 0.036 mg/kg IV (20 mg; Table 1). Oxytetracycline at 7.5 mg/kg IV SID was prescribed but was declined by the team veterinarian at that time in the absence of a definitive diagnosis.

At 1600 hr on July 24, the horse's temperature was 40.4°C. CBC revealed that its PCV was 16.1% (Fig. 1). A blood smear and PCR testing then confirmed a definitive diagnosis of piroplasmosis caused by *Theileria equi* (Figs. 2 and 3). The therapeutic plan was to administer oxytetracycline for 3 days and FM as needed (Table 1). Infected blood cells were counted, and parasitemia (%) was determined, as shown in Table 2. However, the horse showed negative results for anti-*Theileria equi* antibodies in a competitive enzyme-linked immunosorbent assay (cELISA) and IFAT (Table 2).

At 2100 hr, oxytetracycline was initiated at 7.5 mg/kg IV SID (diluted in a liter of isotonic fluid) due to the definitive diagnosis of EP, and FM was continued at 2.2 mg/kg IV BID (Table 1). Additionally, dexamethasone was discontinued due to the definitive diagnosis of infection, as prolonged therapy may have caused immunosuppression and increased the risk of laminitis. A limited number of days had been planned initially to try to stabilize the cell membranes of the limited red blood cell pool to prevent further loss, at least until we could obtain blood for transfusion.

On July 25, hematology revealed a further reduction in PCV to 12.3% (Fig. 1). The horse was moved to the onsite isolation facility due to concerns about public visibility and the exit implications for horses that were returning to Piroplasmosis-free nations but were recently in proximity to a horse with a documented *Theileria equi* infection. An intravenous catheter was placed into the left jugular vein to facilitate immediate access should that become necessary in an emergency. At that time, a press release was issued by the FEI and OIE. In consultation with the Ministry of Agriculture, Forestry and Fisheries (MAFF), the conditions established for the movement of the Olympic horses were to reconfirm that no ticks were present immediately before movement, to use only asphalt roads, and to apply insecticide to the route used once the move was completed.

Two doses of imidocarb dipropionate (Carbesia®; MSD Animal Health, Intervet, France) were secured on July 25 from a team veterinarian. Because of the gastrointestinal complications often associated with imidocarb treatment [11], the horse was treated with scopolamine butylbromide (Buscopan™) 30 min prior to imidocarb administration [1]. Glycopyrrolate and atropine have also been used as pretreatments for imidocarb but were not used for this horse. Dexamethasone (20 mg IV) and FM (1,210 mg IV) were also administered as pretreatments 30 min before imidocarb. Imidocarb was administered intramuscularly (IM; 850 mg, 10 m*l*) and split into equal volumes that were administered to both the left and right sides of the base of the neck. Mild

Table 1. Course of clinical signs, clinical test results, and treatment for the horse with piroplasmosis

Date	Time	History				Treatment		
		Physical examination		Test results		Agent	Amount	Route
July 24	Morning (6:00)	·Lying down and colic		·Complete blood count				
		·Initial examination		Packed cell volume	16.6%			
		Body temperature	40.4°C	Hemoglobin	6.5 mg/d*l*			
		Heart rate	64 beats/min	Red blood cells	3.88×10^6 cells /μl			
		Respiratory rate	20 breaths/min	White blood cells	7.45×10^3 cells /μl			
		·4/6 dystric murmur		Total bilirubin	7.7 mg/d*l*			
		·Gastrointestinal sound was normal		·Splenomegaly				
		·Overnight stool was normal		·Nasal swab				
				EHV-1	Negative			
				Influenza	Negative			
				·Repeat complete blood count				
				Packed cell volume	16.4%			
		Recommended withdrawal				Ketoprofen	2.2 mg/kg	Intravenous (IV)
	9:30					Flunixin meglumine	2.2 mg/kg (1,210 mg)	IV
						Dexamethasone	0.036 mg/kg (20 mg)	IV
	16:00	Body temperature	40.4°C	Packed cell volume	16.1%			
		Definitive diagnosis of piroplasmosis caused by *Theileria equi*						
				·Blood smear + and PCR +				
	21:00					Oxytetracycline	7.5 mg/kg	IV
July 25		Body temperature	37.5°C	Packed cell volume	12.3%	Oxytetracycline	7.5 mg/kg	IV
		Moved to isolation				Scopolamine	0.72 mg/kg	IV
						Dexamethasone	20 mg	IV
						Flunixin meglumine	1,210 mg	IV
						Imidocarb	850 mg 10 m*l*	Intramuscular (IM), both side of neck
July 26		Body temperature	38.5°C	Packed cell volume	19.6%	Oxytetracycline	7.5 mg/kg	IV
						Scopolamine	0.72 mg/kg	IV
						Dexamethasone	20 mg	IV
						Flunixin meglumine	1,210 mg	IV
						Imidocarb	850 mg 10 m*l*	IM, both side of neck
						Whole blood	4 *l*	IV
July 27		Body temperature	38.2°C	Packed cell volume	23.6%	Flunixin meglumine	1,210 mg	IV
						Whole blood	3 *l*	IV

IM swelling occurred at the injection sites within 5–10 min of administration.

On July 26, imidocarb was administered again after pretreatment again with scopolamine, dexamethasone, and FM. Initially, no acute adverse effects of imidocarb were observed; however, on the second administration, the horse pawed briefly as if experiencing some mild abdominal pain, but the signs did not persist for more than 5 min. Whole blood for transfusion was obtained from a JRA universal donor on July 26 (Table 1). The ideal volume of blood to be transfused was calculated as follows in accordance with Sellon and Wise [8].

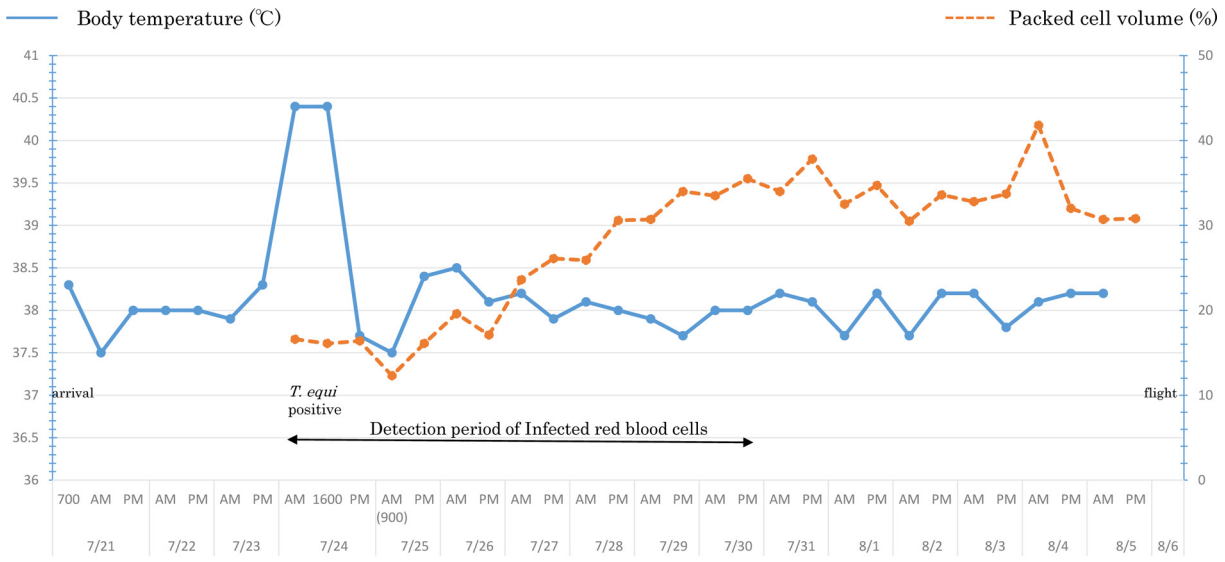

Fig. 1. Changes in body temperature (°C) and packed cell volume (%) during daily measurements.

Body mass (kg) × blood volume (m*l*/kg) × [($PCV_{desired}$−$PCV_{observed}$) / PCV_{donor}]=550 × 72 × [(20−12)/$40_{donor\ estimated}$]=7.9 *l* of donor blood

The total available blood for transfusion was 7 *l* and was split into two transfusion aliquots of 4 *l* (administered IV on July 26) and 3 *l* (July 27, Table 1). Blood was refrigerated during transportation and while awaiting transfusion.

These treatments were effective, as the horse's PCV recovered rapidly to the normal range by July 29, its body temperature stabilized within the normal range, and its general condition clearly improved. The horse did not develop any fevers after July 25, and on August 5, it was considered ready to board its scheduled return flight. The MAFF gave permission for the horse to be returned home. This horse showed negative conventional PCR results for *Theileria equi* genes on the afternoon of August 5, although it had shown positive results from the first day of fever until the morning of August 5 (Table 2). In contrast, IFAT revealed seroconversion against *Theileria equi* (Table 2).

The horse left Japan on an early morning flight on August 6. The MAFF informed the OIE that the outbreak of EP had ended when the isolation stables had been sprayed with acaricides.

The differential diagnoses considered for fever of unknown origin included respiratory diseases, including equine influenza and equine herpes virus (EHV)-1 or 4 infection, pneumonia, and pleuritis. A nasal swab was performed, which was PCR negative on-site for EHV-1 and equine influenza. Anecdotally, a small number of Olympics horses arrived with mild signs of pneumonia after airplane flights of 8,000+ miles but responded quickly to antibiotic and anti-inflammatory therapies. Cardiovascular considerations in the differential diagnosis included phlebitis, endocarditis, anaplasmosis, and EP, while systemic illness concerns included equine infectious anemia (EIA) and red blood cell parasites including EP.

EIA and EP were possible infectious causes of this horse's fever and anemia, so excluding them was essential. However, since the Olympic horses were in quarantine, samples from them were not allowed outside the venue. Therefore, we asked the person in charge of the Animal Health Division of the MAFF (Assistant Director of the international quarantine team) to visit the event venue and obtained consent to transport a blood sample from this horse out of the venue for additional testing.

Considering the relatively long incubation period (12–19 days) of this disease [7, 13], the horse in the present case was thought to have been infected before arriving in Japan. The symptoms developed three days after arrival at the venue, although the horse showed negative results for EP in the pre-import IFATs. Antibodies were not detected until 13 days after the fever, even though red blood cells were infected. A previous study reported that experimental intravenous infection with *Theileria equi* or *Babesia caballi* resulted in positive IFAT results on days 3–20 post infection [13]. Horses infected with *Theileria equi* are detectable by cELISA as early as 21 days after experimental infection and approximately 5 weeks after tick transmission [13]. Therefore, it was crucial not to overlook clinical symptoms that might lead to the suspicion of EP and to create an environment that would allow testing methods that would lead to a definitive EP diagnosis.

Table 2. Summary of hematology, blood chemistry, and laboratory results

	Normal range	July								August					
		24	25	26	27	28	29	30	31	1	2	3	4	5AM	5PM
Complete blood count (CBC)															
Red blood cells (10^6 cells/μl)	6.4–10.4	**3.88**	**3.82**	**4.72**	**5.59**	**6.04**	7.08	7.83	7.81	7.14	6.91	7.35	9.09	6.86	6.95
Packed cell volume (%)	30.0–47.0	**16.6**	**12.3**	**19.6**	**23.6**	**25.9**	30.7	33.5	34	32.5	30.5	32.8	41.8	30.7	30.8
Hemoglobin (g/d*l*)	10.7–16.5	**6.5**	**6.4**	**7.9**	**9.3**	**10.2**	12	13.2	13.4	12.3	11.9	12.7	15.4	11.7	11.2
Mean corpuscular volume (f*l*)	41.1–52.4	42.8	42.1	41.5	42.2	42.9	43.4	42.8	43.5	45.5	44.1	44.6	46	44.8	46.7
Mean corpuscular hemoglobin (pg)	14.1–18.6	16.8	16.8	16.7	16.6	16.9	16.9	16.9	17.2	17.2	17.2	17.3	16.9	17.1	17
Mean corpuscular hemoglobin concentration (g/d*l*)	32.8–38.6	**39.2**	**39.8**	**40.3**	**39.4**	**39.4**	**39.1**	**39.4**	**39.4**	37.8	39	38.7	36.8	38.1	36.4
White blood cells (10^3 cells/μl)	4.90–11.10	7.45	**11.96**	9.64	8.12	7.35	7.82	7.66	7.57	7.65	7.7	7.57	5.02	5.97	6.58
Neutrophils (10^3 cells/μl)	2.50–6.90	4.71	**8.64**	6.9	5.63	4.31	5.21	5.14	4.5	4.66	5.09	4.74	3.06	3.52	3.86
Lymphocytes (10^3 cells/μl)	1.50–5.10	1.55	1.81	1.57	1.60	2.15	1.99	2.00	2.37	2.48	2.23	2.48	1.71	2.11	2.30
Blood chemistry															
Glucose (mg/d*l*)	64–150	124	138	ND	181	148	ND	ND	ND	ND	ND	ND	ND	87	ND
Creatinine (mg/d*l*)	0.8–2.2	1.4	0.9	ND	1.3	1.5	ND	ND	ND	ND	ND	ND	ND	1.6	ND
Blood urea nitrogen (mg/d*l*)	10–25	18	21	ND	11	12	ND	ND	ND	ND	ND	ND	ND	19	ND
Calcium (mg/d*l*)	10.4–12.9	10.5	11.4	ND	12.4	12.3	ND	ND	ND	ND	ND	ND	ND	11.0	ND
Total protein (g/d*l*)	5.6–7.9	6.4	6.1	ND	6.7	6.7	ND	ND	ND	ND	ND	ND	ND	6.5	ND
Albumin (g/d*l*)	1.9–3.2	2.8	2.6	ND	3.0	3.0	ND	ND	ND	ND	ND	ND	ND	2.7	ND
Globulin (g/d*l*)	2.4–4.7	3.6	3.4	ND	3.7	3.8	ND	ND	ND	ND	ND	ND	ND	3.7	ND
Aspartate aminotransferase (U/*l*)	100–600	237	204	ND	283	303	ND	ND	ND	ND	ND	ND	ND	224	ND
Alanine aminotransferase (U/*l*)	10–326	132	168	ND	197	203	ND	ND	ND	ND	ND	ND	ND	160	ND
Ganma-glutamyl transpetidase (U/*l*)	0–87	19	19	ND	26	26	ND	ND	ND	ND	ND	ND	ND	23	ND
Total bilirubin (mg/d*l*)	1.1–3.5	**7.7**	**7.3**	ND	3.2	2.1	ND	ND	ND	ND	ND	ND	ND	1.3	ND
Creatine kinase (U/*l*)	10–350	67	27	ND	198	216	ND	ND	ND	ND	ND	ND	ND	106	ND
Lactate dehydrogenase (U/*l*)	250–2,070	1,173	764	ND	870	1,079	ND	ND	ND	ND	ND	ND	ND	1,238	ND
Laboratory results															
Infected RBC (cells/μl)			**141 × 10^2**	**71 × 10^2**	**47 × 10^2**	**36 × 10^2**	**Too few**	**Too few**	ND	ND	ND	ND	ND	ND	ND
Iinfected RBC/total RBC (%)			**0.25**	**0.07**	**0.09**	**0.07**	ND	ND	ND	ND	ND	ND	ND	ND	ND
Conventional PCR		+	+	+	+	+	+	+	+	+	+	+	+	+	-
cELISA		–	–	–	–	–	–	–	–	–	–	–	–	–	–
IFAT		–	–	–	–	–	–	–	–	–	–	–	–	–	+(1;80)

ND, not determined; −, negative; +, positive; cELISA, competitive enzyme-linked immunosorbent assay; IFAT, indirect fluorescent antibody test. Abnormal findings are indicated in bold type.

In addition, there was almost no risk of infection because hygiene management was implemented at the venue. In Japan, *Dermacentor reticulatus* and *Rhipicephalus sanguineus* ticks have the ability to carry the disease. However, tick surveys and insecticide treatments were conducted at Bajikoen for five years before the Olympic Games, and it was confirmed that there were no ticks inhabiting the venue just before the arrival of the horses [2]. In addition, since none of the horses that came to Japan to participate in the Olympic equestrian events had contact with domestic horses, there was no risk of transmission of this disease to domestic horses.

Imidocarb dipropionate is a carbanilide derivative with antiprotozoal activity [6, 7, 11]. However, two doses of imidocarb appeared to be insufficient to kill all the parasites. Should infected horses recover from this clinical disease,

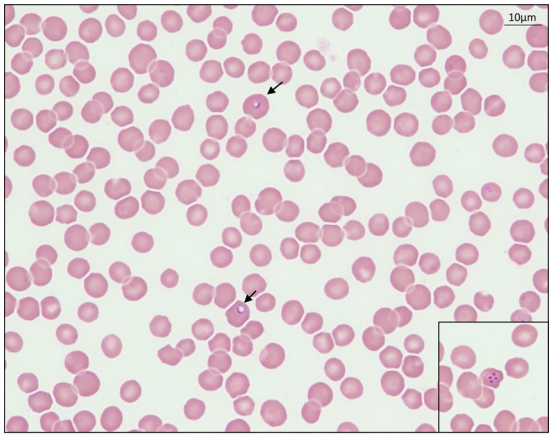

Fig. 2. May-Grunwald Giemsa staining of red blood cells containing intraerythrocytic inclusions of *Theileria equi.* The arrows show inclusions of *Theileria equi*. Typical Maltese crosses were also seen occasionally (insert).

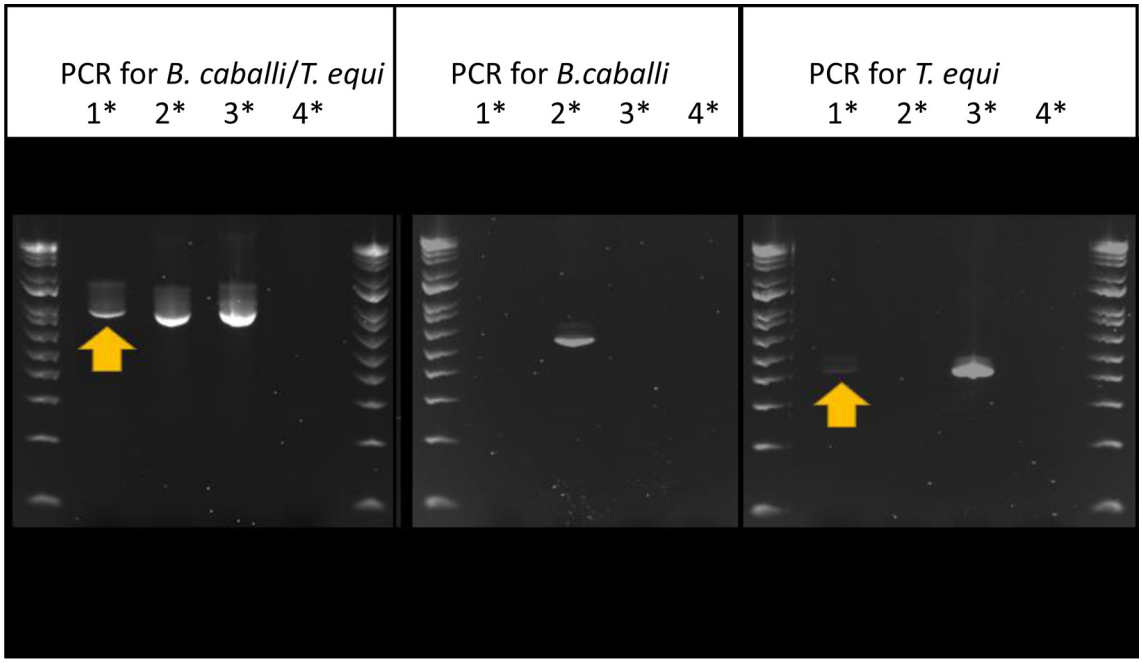

Fig. 3. Direct PCR showed that the patient's blood sample was positive for *Theileria equi.* Yellow arrows indicated positive PCR results for *Theileria equi* in the patient's blood. *[1]Patient blood. *[2]Positive control for *Babesia caballi*. *[3]Positive control for *Theileria equi*. *[4]Negative control.

they may become carriers of *Theileria equi* infection for several years, with the parasites persisting in many of their organs, including the bone marrow, liver, spleen, lungs, heart, and brain [10]. In this case, the antibody titer in this horse increased, as determined by the IFAT on day 13 (August 5) only a few hours before returning home; however, the horse showed negative results for *Theileria equi* genes in PCR testing. In infected horses that survive the acute phase, the blood parasites may be retained for years.

In conclusion, during the Tokyo 2020 Olympic Games, we successfully managed an acutely ill EP-affected horse that had been imported as antibody negative from Germany. The affected horse was returned home without transferring the disease to other competing horses.

Since cases that cannot be proven to be positive by inspection may be imported, daily health management of horses should be accompanied by accurate and prompt information dissemination and tick inhabitation quarantine measures, including investigation and extermination. Prevention of EP requires eradication of vector ticks, detection of infected horses and carriers by antibody reaction, isolation of positive horses, and appropriate treatment.

References

1. Abutarbush, S.M., Alfaqeeh, S.M., Mustafa, G., Qura'n, L., and Al-Majali, A.M. 2013. Evaluation of the use of atropine sulfate, a combination of butylscopolammonium bromide and metamizole sodium, and flunixin meglumine to ameliorate clinical adverse effects of imidocarb dipropionate in horses. *Am. J. Vet. Res.* **74**: 1404–1408. [Medline] [CrossRef]
2. International Animal Health Affairs Office Animal Health Division, Ministry of Agriculture, Forestry and Fisheries. 2021. Hygiene management consignment business at the Tokyo 2020 Olympic and Paralympic Games equestrian stadium. Business summary from 2016–2021 (in Japanese). https://www.maff.go.jp/j/budget/yosan_kansi/sikkou/tokutei_keihi/seika_R03/attach/pdf/itaku_R03_ippan-611.pdf [accessed on May 1, 2022].
3. International Animal Health Affairs Office, Animal Health Division Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries of Japan. 2021. Self-declaration of an Equine Disease-Free Zone in Tokyo, Japan, for the purpose of facilitating the Equestrian competitions of Tokyo 2020 Olympic and Paralympic Games. https://www.woah.org/app/uploads/2021/07/2021–06-japan-edfzannexes.pdf [accessed on May 1, 2022].
4. Kappmeyer, L.S., Thiagarajan, M., Herndon, D.R., Ramsay, J.D., Caler, E., Djikeng, A., Gillespie, J.J., Lau, A.O., Roalson, E.H., Silva, J.C., Silva, M.G., Suarez, C.E., Ueti, M.W., Nene, V.M., Mealey, R.H., Knowles, D.P., and Brayton, K.A. 2012. Comparative genomic analysis and

phylogenetic position of *Theileria equi*. *BMC Genomics* **13**: 603. [Medline] [CrossRef]
5. Nicholson, W.L., Sonenshine, D.E., Noden, B.H., and Brown, R.N. 2019. Medical and veterinary entomology. pp. 603–672 *In:* Ticks [Ixodida], Elsevier, Amsterdam.
6. Onyiche, T.E., Suganuma, K., Igarashi, I., Yokoyama, N., Xuan, X., and Thekisoe, O. 2019. A review on equine piroplasmosis: epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. *Int. J. Environ. Res. Public Health* **16**: 1736. [Medline] [CrossRef]
7. Rothschild, C.M. 2013. Equine piroplasmosis. *J. Equine Vet. Sci.* **33**: 497–508. [CrossRef]
8. Sellon, D.C., and Wise, N. 2010. Disorders of the hematopoietic system. p. 766. *In*: Equine Internal Medicine, 3rd ed. (Reed, S.M., Bayly, V.M, and Sellon, D.C. eds.), Sanders Elsevier, St. Louis.
9. Tirosh-Levy, S., Gottlieb, Y., Fry, L.M., Knowles, D.P., and Steinman, A. 2020. Twenty years of equine piroplasmosis research: global distribution, molecular diagnosis, and phylogeny. *Pathogens* **9**: 926. [Medline] [CrossRef]
10. Tuvshintulga, B., Nugraha, A.B., Mizutani, T., Liu, M., Ishizaki, T., Sivakumar, T., Xuan, X., Yokoyama, N., and Igarashi, I. 2021. Development of a stable transgenic Theileria equi parasite expressing an enhanced green fluorescent protein/blasticidin S deaminase. *Sci. Rep.* **11**: 9107. [Medline] [CrossRef]
11. Ueti, M.W., Mealey, R.H., Kappmeyer, L.S., White, S.N., Kumpula-McWhirter, N., Pelzel, A.M., Grause, J.F., Bunn, T.O., Schwartz, A., Traub-Dargatz, J.L., Hendrickson, A., Espy, B., Guthrie, A.J., Fowler, W.K., and Knowles, D.P. 2012. Re-emergence of the apicomplexan *Theileria equi* in the United States: elimination of persistent infection and transmission risk. *PLoS One* **7**: e44713. [Medline] [CrossRef]
12. Veterinary Medication. 2023 Veterinary Regulation, 15th ed., effective 1 January 2023. p. 49. Federation Equestre Internationale. https://inside.fei.org/content/fei-veterinary-rules [accessed on May 1, 2022].
13. Wise, L.N., Kappmeyer, L.S., Mealey, R.H., and Knowles, D.P. 2013. Review of equine piroplasmosis. *J. Vet. Intern. Med.* **27**: 1334–1346. [Medline] [CrossRef]
14. Zobba, R., Ardu, M., Niccolini, S., Chessa, B., Manna, L., Cocco, R., and Parpaglia, M.L.P. 2008. Clinical and laboratory findings in equine piroplasmosis. *J. Equine Vet. Sci.* **28**: 301–308. [CrossRef]